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Biomining of Chromium Ions from Chrome Ore of Muslim Bagh, Balochistan

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Abstract

Chromite ore was collected from Muslim Bagh, Pakistan and was ground to powder form with uniform size. For biomining, bacteria were isolated from two different samples obtained from coal mine water (Quetta) and wastewater (Islamabad). The microbes were cultured in 9K media. The powdered ore was added in an acidic solution whose pH was 2.5, to acidically dissolve the ore. The acidic solution was then filtered and titrated against standardized Ferrous Sulfate (FeSO4) to check the acidic dissolution of the ore. However, no reasonable outcome was observed. The ore was then suspended into 9K media having sulphur or FeSO4 with bacterial inoculation. The ore was subjected to bacterial activity up to 22 days after inoculation. The Chromium content, dissolved by the bacteria in the solution, was determined through titration as well as atomic absorption spectroscopy at different intervals i.e. 14 and 22 days after inoculation respectively. Titration produced no results. However, the outcome of atomic absorption spectroscopy manifested Cr content dissolved in the solution up to 0.687 mg/l from 1g of chromite ore after 22 days of bacterial activity. If the ore subjectivity to bacteria is extended, it might produce better results.

Keywords: Biomining; Acidophilic bacteria; Chromite ore; Muslim Bagh; Atomic absorption spectroscopy

Introduction

Biomining is the process of extracting minerals from their ores through the metabolic action of microbes including certain species of fungi such as Aspergillus niger and Acidithiobacillus species of bacteria such as Acidithiobacillus ferrooxidans etc. Biomining is more instrumental in the bioleaching of sulfidic ores that contain sulfides in its chemical composition. Bioleaching or biomining is a natural process where basically microorganism facilitates the extraction of a mineral by creating acidic condition i.e. by the production of sulfuric acid. Biomining was an ancient and unnoticed process until it came to limelight as a phenomenon in the 1950s after the discovery of acidophilic microbes. Today around 20% of copper and 5 % of gold is extracted through the process of biomining. Other metals such as nickel, zinc etc. are produced in small quantities through biomining. Microorganisms accomplish the process of biomining by either oxidizing the desired metal and thus dissolving it into the solution directly like copper from chalcopyrite ore or oxidizing the surrounding atoms (Fe and As) in the mineral compound e.g. arsenopyrite and making the desired metal such as gold more accessible for extraction and isolation. These two procedures of biomining are known as bioleaching and bio-oxidation respectively. Generally mineral should have iron or reduced form of Sulphur. The microbes grow by oxidizing reduced Sulphur or ferrous iron or both. Role of microbe is to produce ferric iron and H2SO4 which is the basic requirement of mineral degradation. Acidithiobacillus spp oxidize Sulphur and metal sulphide to sulfuric acid and metal sulfate while ferrous iron to ferric iron which is also a catalyst for metal solubilization:

The microbes used for biomining have distinct characteristics such as they are acidophiles (grow at pH 1.4 to 1.6), metal and thermal resistant (operate between ambient temperature to 40°C), and grow autotrophically. Commercially biomining is conducted by putting mineral ore in heap or dump reactors where it is irrigated or minutely milled mineral ore is placed in a stirred tank and vigorously aerated at a temperature between 40-55 °C. Currently many countries including Finland, Chile are switching towards biomining to extract minerals from mineral waste and other mineral reserves because it is more economical and environmentally friendly than conventional mining procedures. However, it is more time consuming than conventional procedures.

Biomining of chromium can play a very vital role in remediating the toxic concentration of Chromium especially chromium-VI which is very carcinogenic and can cause serious health and environment issues. Industries like electroplating, cement and leather tanning are the major source of releasing toxic chromium into the environment. This release of chromium waste in the environment causes the accumulation of high concentration of chromium in water streams and reservoirs such as river and lakes respectively and as well as affect the surrounding plant and animal life and their habitat. Different techniques and methods are used for the treatment of and reducing the toxic concentration of chromium in the environment. These techniques are distributed on a wide range of physical and chemical processes. The problems with the usage of such techniques are the high cost associated with its operational mechanism, sludge formation which requires additional treatment procedures as well as certain complications that arises in the management of these techniques. These all problems can be effectively addressed using microbial organisms as bio sorbents and reducers that accumulates waste within their cellular boundaries and ultimately reduced the waste into a non-toxic or less

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toxic by-product through the action of their enzymatic and metabolic machinery. The biological remediation of certain toxic chemicals from the environment is more economical, effective and environmentally friendly than other conventional methods. Using microbes that too of an indigenous environment prone to the pollution of chromium can help to reduce the toxic concentration of Cr-VI to a negligible level. Microbes use their metabolic machinery to reduce chemical components that are considered as a toxic material.

Chromium is present in chromite ore which is an iron chromium oxide with formula $FeCr_2O_4$. In Pakistan, major reserves of chromite are present in ultramafic rocks of Muslim Bagh and Khanozai, Balochistan.

Several studies suggest that biomining is an effective process for the extraction of metals from their mineral ores. It is a promising process for sulfidic ores. The major metals extracted throughout the world using biomining are copper and gold. However, other metals are also extracted through biomining which include nickel, iron, chromium, manganese, and zinc etc. Copper and gold are present in minerals which are sulfidic like chalcocite, chalcopyrite and arsenopyrite respectively. In the US in 1978, 18% of total copper production was through bioleaching. In Pakistan copper was extracted through the process of bioleaching from chalcopyrite ore in 1988. Acidithiobacillus thiooxidans NB101 and Acidithiobacillus ferrooxidans NB102 were used for bioleaching of copper. Sulphur was added as an energy source and pH was maintained at 2.5. After 14 days of culture, up to 19% of copper was solubilized from chalcopyrite. The basic chemical reaction involves bioleaching is given below:

MS (metal sulphide) + H_2SO_4 + $0.5O_2$ → MSO_4 + H_2O + S

Conventional leaching methods are primarily used for the extraction of chromium from its ore present in the earth crust or chromite ore processing residue (COPR) resulted from the lime processing of chromite ore and contain a high quantity of Cr (III and VI). Such as the addition of Ferrous Sulfate which reduces the Cr (VI) into Cr (III) and subsequently precipitates Iron chromium hydroxide ($CrFeH_5O_5$). Ferrous sulfate enhances the Cr (VI) leaching and helps in the remediation of chromium from the environment.

Use of microbes for bioleaching is mainly used for the extraction of Cr residuals from wastewater and tanneries effluents. Cr (VI) exists in these wastewater and industrial effluents which are highly cancerous and can cause severe health problems in humans and other living organisms. In third world countries like Pakistan, this problem becomes more severe as these industrial effluents are not filtered properly before delivering it to sewage water which ultimately enters the rivers and canals that is used for irrigation and human consumption because of lack of government regulation and supervision. Microbial leaching was used for reducing the concentration of Cr^{+6} in chromium-containing tanneries effluents near Kasur, Pakistan. The microbial consortium that comprised of protozoa and bacteria was found to decrease all the Cr^{+6} present in the original sample of tanneries effluents within one week. In another study conducted in 2010, a bacterial Bacillus specie strain (CSB-4) isolated from the Indian chromite mine soil was used to reduce the concentration of hexavalent chromium. The strain was found to be capable of reducing Cr (VI) to Cr (III) in different growth media. The bacterial strain reduced more than 90% of hexavalent chromium. Following the reduction of hexavalent chromium, Cr (III) is then isolated through the process of precipitation and thus the toxic level of Cr (VI) in wastewater or mining sites can be reduced.

The most important bacteria responsible for the bioleaching of metals especially those contain iron in their composition is the A. ferrooxidans which has the capability of oxidizing the Fe^{2+} present in the ore to Fe^{3+}. Chromite ore also contains Fe in its chemical structure, so A. ferrooxidans can be used to oxidizing out the iron which will break the chemical skeleton of chromite and thus separating the chromium from its ore can be possible. This is an indirect way of extracting chromium from its ore also known as bio-oxidation.

Biomining is economically preferable than conventional mining procedure and currently, it is being used for the processing of reduced ores and mineral wastes. Iron-containing minerals that possess Ni, Co, Cr and Mn can be effectively solubilized. A study was conducted on microbial extraction of nickel from lateritic chromite overburden (COB) in Sukinda, India using Acidithiobacillus ferrooxidans in an anoxic environment which successfully reduced ferric iron in goethite [Fe(O)OH] mineral of COB by the oxidation of elemental sulphur which acted as an electron donor. At the same time, 41% nickel was also recovered from the complex goethite matrix of COB due to the action of sulphuric acid generated by the oxidation of elemental sulphur a reduction of ferric iron in a 3 L scale bioreactor at pH of 1.8 ± 0.05, temperature of 28 ± 2 °C and was maintained in an anoxic environment. A similar study was conducted in which A. ferrooxidans was used to solubilize the nickel and other metals such as Cr, Co and Mn effectively at 30 °C and was maintained in solution at 1.8 pH through the reduction ferric iron and oxidation of elemental sulphur in goethite fraction of limonitic nickel ore.

Chromite ore processing residue (COPR) is a waste product generated during the industrial production of chromate that is the major source of Cr-VI contamination in the environment. So, a bacterial specie Pannonibacter phragmitetus BB was used to remediate Cr-VI from these residues. Water-soluble Cr (VI) of COPR-A and B is 3,982.9 and 1,181.4 mg/kg, respectively were treated. It was observed that Pannonibacter phragmitetus BB reduced Cr-VI in leachate to a negligible level at the flow rate of 1 and 2ml/min in column biotreatment process. While in, direct biotreatment process Cr (VI) in the liquid supernatant of COPR-A and B decreased from 265 and 200 mg/l to 145 and 40 mg/kg after 240 hrs of incubation.

Bioleaching technique was used for the improvement of the Cr/Fe ratio of Turkish chromite concentrate. Bacterial leaching for iron removal was investigated from chromite concentrate obtained from Kosbucagı, Mersin, Turkey. Acidithiobacillus ferrooxidans were used in this leaching process. The dissolution of different solid rates was observed on the shake-flask. The highest iron (Fe) extraction value was determined as 14.10% at 3% solids and the Cr_2O_3 content of the chromite concentrate was upgraded to 51.50% from 48.70% with Cr/Fe ratio of 2.73 from 2.24.

Chromium hexavalent and its several compounds have been characterized as carcinogenic, mutagenic and teratogenic. There are several methods and techniques are used for the treatment of Cr-VI and reducing its concentration in the environment. It includes reduction of Cr concentration with iron and sulphur-based chemicals, thermal treatment of COPR and biological treatment of COPR. Biological treatment exploits microorganisms and their enzymatic activity to reduce Cr in the solution and immobilize it. The microbial reduction of chromium is achieved in highly alkaline systems. For example, Leucobacter specie, gram-negative bacteria isolated from a COPR site in China, reduced approximately 2500 mg/l chromate to Cr-III in 17 hrs.

Chromium-VI high concentrations in the environment and water streams due to mineral waste pose a very serious threat to the environment and ecosystem and its reduction to Cr-III and its subsequent removal from the environment is need of the hour. Several techniques are being studied and used to cope with this environmental menace. But of all the techniques, bioleaching is considered to be the most plausible solution as it uses indigenous bacterial species to remediate Cr-VI which is pretty much natural and economical process. For this purpose, a chromate resistant bacterial strain Cr8 was isolated from chromite slag identified as Pseudomonas stutzeri. P. stutzeri Cr8 reduced Cr (VI) at initial concentrations of 100 and 200 mg L−1 Cr (VI) completely within 24 hrs, whereas it reduced almost 1000 mg L−1 Cr (VI) at the end of 120 hrs. Moreover, soil column leaching experiments were performed which revealed that bacterial cells reduced Cr (VI) leachate at a faster rate and that almost disappeared at the end of 168 hrs.

Material & Methods

Chromite ore Sample Collection

The chromite ore sample was collected from the chromite mines of Muslim Bagh district Killa Saifullah of Balochistan, Pakistan. The ore sample was ground to powder form with uniform size using a sieve.

Media Preparation

200 ml 9K Media was prepared which is suitable for the growth of iron-oxidizing bacteria such as Acidithiobacillus ferrooxidans. Its pH was adjusted at 2.5 by adding H2SO4 solution dropwise. After autoclaving the media, it was distributed in four flasks about 50 ml in each flask. Then 5ml FeSO4 solution was added to the first two flasks and 0.5g Sulphur in other two flasks. All four flasks were inoculated with 5ml samples obtained from different sources separately.

Bacterial isolation and identification

Different samples were used for the isolation of iron-oxidizing bacteria that includes samples from Chitral Hot spring, common university wastewater, chromite mine water obtained from different chromite mines located in Muslim Bagh, coal mine water from two different regions of Balochistan. Growth only appeared in samples of coal mine water from Balochistan and wastewater obtained from Islamabad when the samples were inoculated in 9K media in flasks. The flasks were placed in shaking incubator at 37°C and 130 rpm. It was observed that the initial pH (2.5) of media was dropped to 1.4 and 1.5 in flasks inoculated with 5ml coal mine water and 5ml wastewater respectively by checking the changes in pH of flasks periodically. The bacterial growth was confirmed when observed under a microscope.

Chemical Solubilization of Chromite

For chemical solubilization of chromite ore, 300ml acidic solution was prepared and its pH was adjusted at 2.5 by the addition of H2SO4 solution dropwise. The 300ml solution was distributed in four flasks about 50ml in each. The ore was added in each flask with different concentration such as 0.1g, 0.2g, 0.5g and 1.0g respectively. The four flasks were placed at shaking incubator for 2 hours.

Titration of Standardized FeSO4 solution

Standardized FeSO4 solution was titrated against the potassium dichromate solution. Titration was repeated for three times to get three different readings. Diphenylamine indicator was used for the measurement of change in colour.

Titration of Potassium dichromate solution

Potassium dichromate solution was titrated against the standardized FeSO4 solution. Different concentrations of K2Cr2O7 with two drops of di-phenyl amine indicator in each concentration were used to determine the endpoint.

Titration of Acidic solution of Chromite ore

The acidic solution of chromite ore was filtered and titrated against standardized FeSO4 solution. The concentration of standardized FeSO4 solution includes 0.1M FeSO4 solution, H2SO4 solution and (1:1) phosphoric acid solution. Diphenyl Amine indicator was used to measure the change in colour and figure out the endpoint. No change in colour was observed.

Biological Degradation of Chromite Ore

For biological degradation of chromite ore powder through the metabolic action of Acidithiobacillus ferrooxidans, it was added with a concentration of 1g ore powder in every four flasks containing 50ml 9K media. Each flask was inoculated with 5ml bacterial solution. 5ml FeSO4 solution and 0.5g Sulphur was added in the first two and last two flasks respectively. A control solution was also prepared in two flasks having 50ml 9K media and 1g ore powder in each flask. 5ml FeSO4 and 0.5g Sulphur were added in two flasks containing control solution respectively.The flasks were then placed in shaking incubator at 30°C for two weeks. During the shaking period, the pH of the flasks was periodically checked, and an increase was observed from its initial 2.5pH. The bacterial growth still appeared in the solution when checked under a microscope. At different intervals i.e. 14 days and 22 days after inoculation of the samples, each time around 10ml solution was filtered from each flask for chemical analysis of chromium through titration and atomic absorption spectroscopy for precise results.

Titration of filtered Solution

The bacterial solution containing ore and control solution was filtered to chemically analyse it through titration. The filtered solution was titrated against the standardized FeSO4 solution. Diphenyl Amine indicator was used for measuring the endpoint.

Results

Analysis of the Samples used for Microbial isolation

Various samples including Chitral Hot spring, common university wastewater, chromite mine water, Islamabad wastewater and coal mine water. Growth only appeared in samples of Islamabad wastewater and coal mine water. The pH of both the samples was checked peri2odically and a considerable decline in the pH from the initial pH i.e. 2.5 was observed (table 1 & 2)(figure 1).

Table 1: Periodic pH of samples containing Wastewater for Bacterial Isolation

Wastewater Sample (Islamabad)	Initial pH on (09/05/2018)	pH on (16/05/2018)	pH on (04/06/2018)	pH on (26/06/2018)
W. W+9K media+FeSO4	2.5	1.94	1.75	1.5

Table2: Periodic pH of samples containing coal mine water and bacteria

Coal mine water sample (Hana, Balochistan)	Initial pH (16/05/2018)	pH on (04/06/2018)	pH on (26/06/2018)
C.M. W+9K media+FeSO4	2.5	1.60	1.49

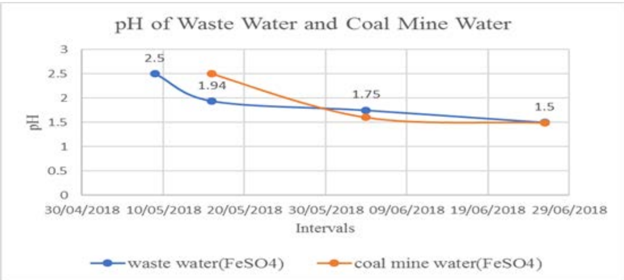

Figure 1: Graph displaying pH variation in wastewater and coal mine water samples used for isolation of bacteria

pH of Samples containing Chromite ore and Bacteria

The pH of samples containing chromite powdered ore and bacteria was analyzed periodically. An increase was observed in the pH from the initial pH i.e. 2.5 of these flasks. However, bacterial growth still appeared in the samples(figure 2 & 3).

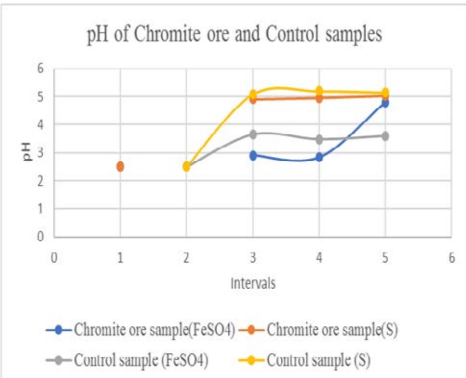

Figure 2: Graph displaying pH variation Chromite ore inoculated with bacteria and Control samples

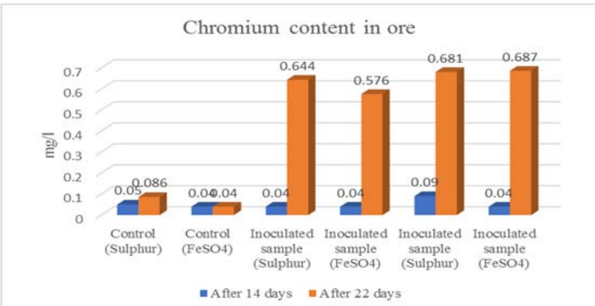

Figure 3: Graphical representation of Cr content in Chromite ore

pH of Control Sample

The pH of Control sample, with powdered chromite ore and 9K media that have not been inoculated with bacterial culture, was also checked periodically to observe the change in pH.The pH of the sample increased from its initially adjusted pH i.e. 2.5.

Titration of Acidic Chromite Ore Solutions

Acidic solutions of Chromite ore were filtered, and the filtered solutions were titrated against standardized FeSO4 solution separately. However, no change in colour was observed and no endpoint was determined during the titration process. Thus, only acid treatment (pH 2.5) did not solubilize chromium.

Determination of Chromium concentration through Atomic absorption spectroscopy of Bacterially Treated Ore in Solutions

For the precise determination of chromium concentration in the chromite ore solubilized by the bacterial metabolic system, around 10ml solution was filtered from each flask inoculated with bacterial culture and from control flasks at different intervals i.e. 14 and 22 days after inoculation respectively. The filtered samples were analysed to determine the chromium content in the ore through atomic absorption spectroscopy. Following statistics were obtained during the process.

Discussion

Biomining of ore using metal oxidizing bacteria that extract metal from its ore is a very effective procedure and can play a major role in future endeavours in the field of Extractive Metallurgy. Acknowledging the importance of biomining, chromite ore was subjected to the action of metal-reducing bacteria to extract the chromium from the ore. The ore was obtained from the chromite mines of Muslim Bagh, Balochistan, Pakistan. The ore was ground to powder form with uniform size. The metal-reducing bacteria i.e. Acidithiobacillus ferrooxidans, which was isolated from samples of coal mine water and wastewater was used for the biomining activity. Similarly, isolation of these bacteria was earlier reported from wastewater. During the process of biomining, the ore was suspended in flasks containing 9K media with bacterial inoculation and was placed in shaking incubator at 30°C for 22 days. Titration was used to analyse the chromium content extracted from the ore in the solution. However, the titration process yielded no results. Thus, atomic absorption spectroscopy was used to get the precise outcome of the biomining process. Samples analysed through atomic absorption spectroscopy were taken at different intervals i.e. 14 and 22 days respectively after the inoculation. The aforementioned results produced by atomic absorption spectroscopy shown that a certain amount of chromium has been extracted from the ore due to the action of bacteria. Though the samples analysed were subjected to bacterial activity for just 22 days but still, the chromium content being solubilized shows the successfulness of the process.

Conclusion

This paper has manifested the successful extraction of chromium metal ions from its mineral ore through the metabolic action of Acidithiobacillus ferrooxidans, which is an iron-oxidizing bacterium. The data also suggests that if the subjected bacterial action is prolonged and more time is given to bacteria to extract chromium from the ore, then the outcome of the biomining might improve and more Cr might be solubilized from ore.

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